28. A fragment of a polypeptide that is obtainable by recombinant expression of the DNA of HHV-8 in an isolated cell and which comprises the amino acid sequence of SEQ ID NO:2.

34. A method of culturing cells using v-IL-6.

35. The method of claim 34, wherein said cells are selected from the group consisting of lymphocytes, hybridomas, hemopoietic and endothelial cells.

## REMARKS

Applicants and their attorney thank the Examiner for reviewing the claims on October 2nd. During that discussion the Examiner suggested several changes to put claims in condition for allowance. Applicants believe that the present amended claims include these changes and request reconsideration.

Upon entry of the foregoing amendment, claims 1-6, 8-12, 16, 18-20, 28 and 34-35 are pending and claims 7, 13-15, 17, and 29-33 are canceled. Marked up copies of the amended claims and the specification accompany this response.

### Objection to the Specification

On page 2 of the June 20, 2001 Office Action, the Examiner objected to the specification in view of several informalities. In response, applicants have changed a section heading as suggested and made other changes to the specification as requested in the 10/3/00 Office Action.

The term "b1" has been altered to "b."

The clause "shall be comprised" has been removed and replaced with clearer language.

Applicants point out that the phrase "v-IL-6 or the polypeptide" intends to mean both in the alternative, and that the term polypeptide is explained earlier in the paragraph. The spelling of the term "auxilliary" has been amended to "auxiliary."

The spelling of the term "hemopoetic" has been amended to "hemopoietic."

The name "california" has been capitalized.

The reference to the Dayhoff criteria has been modified in the figure legend for Figure 2. Applicants point out that skilled artisan's understand the "Dayhoff criteria." For example, a tutorial on protein sequences found in www.psc.edu/biomed/TUTORIALS/SEQUENCE/DBSEARCH/oldtutorial.html refers to the "Dayhoff Model" and "Dayhoff Family" in this context on page 12.

Reconsideration and allowance are requested.

Claim Objections

The Examiner has pointed out misspelling of the word "competively" in claim 8. This spelling has been corrected.

Rejections under 35 U.S.C. § 101

The Examiner suggested adding the term "in an isolated cell" to overcome this rejection. The suggested language was added to claims 1, 2, 9, 10, and 28.

Rejections under 35 U.S.C. § 112, first paragraph:

The Examiner has rejected claims 4-6, 8, 12, 18, 19, 34 and 35 under 35 U.S.C. 112, first paragraph, as containing subject matter not described in the specification. As explained during the interview, the fragments recited in claims 4-6 include SEQ ID NO: 2. Claim 7 has been cancelled. Claim 8 has been amended to recite a condition that "the fragment binds to the receptor." Claim

12 has been amended to recite a condition that "the nucleic acid codes functional v-IL-6."

Reconsideration and allowance in view of the amendments are requested.

As suggested by the Examiner, the terms "pharmaceutical" and "which may be used in treatment" have been removed from claims 18 and 19. Reconsideration and allowance are requested.

As suggested, the terminology of "used in treatment" has been removed from claims 34 and 35. Reconsideration and allowance are requested.

Rejections under 35 U.S.C. § 112, second paragraph:

The Examiner has rejected claims 1, 2, 4-6, 8-12, 16, 18-20, 28, 34 and 35 as indefinite under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter as the invention.

Claims 1, 2, 9, 10, 28 and claims that depend thereon have been clarified by reciting that the v-IL-6 is obtained "in an isolated cell."

Claims 2, 3, 10 and 28 are rejected because "there is no amino acid sequences set forth in Figure 2."

In response, applicants amend the drawings by labeling the first page of Figure 2 2A, the second page 2B and the third page 2C. Applicants further amend these claims by adding SEQ ID NO as appropriate.

Claims 4 and 5 are rejected because of the use of upper case and lower case letters. In response all of the letters have been amended to upper case lettering.

Claim 7 has been cancelled, mooting the rejection of this claim.

Claim 8 has been clarified by incorporating the features "binds to the IL-6 receptor" and "wherein the fragment binds to the receptor" into this claim.

Claim 11 has been amended to recite SEQ ID NO 1.

Claim 12 has been clarified by the recitation "wherein the nucleic acid encodes functional v-IL-6."

Claim 16 has been rejected for reciting only one component of the test kit. Applicants point out that test kits are well known, a most important feature of the claimed test kit is a nucleic acid that consists of SEQ ID NO:1, and the term "comprising" indicates that other components may be used.

Claim 18 now refers to a polypeptide of claim 2 that has been more clearly defined by amendment to claim 2.

Claim 34 has been clarified by removal of the phrase "which may be used in treatment, comprising adding to a cell culture comprising said cells a cell growth-stimulating amount of."

Claim 35 has been clarified by correcting a spelling error for "hemopoietic."

Rejections under 35 U.S.C. § 102(a), (b):

Claims 9-12 and 16 are rejected as being anticipated by Zhong and Chang. The Examiner pointed out that this rejection could be overcome by amending the phrase "consisting essentially of" to "consisting of" at the top of page 10 of the June 20, 2001 office action. The word "essentially" has been removed and the amended claims now recite "consisting of the sequence of SEQ ID NO:1 and coding for v-IL-6, which is obtainable by recombinant expression of the DNA of herpes virus type-8."

Reconsideration and allowance are requested.

CONCLUSION:

In view of the foregoing, Applicants respectfully request the Examiner to withdraw each rejection and pass the claims on to allowance. The Examiner is invited to contact the undersigned attorney to resolve any issues, in order to expedite the prosecution of the application.

Respectfully submitted,

December 20, 2001
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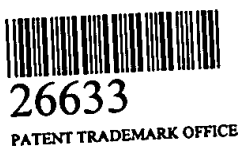

**Version with markings to show changes made**

Page 7, lines 15 to 25: Delete these lines, which begin with "Legends:" and end with "Nucleic acid sequence encoding v-IL-6 and corresponding amino acid sequence."

Page 2, between lines 3 and 4, immediately prior to "DETAILED DESCRIPTION OF THE INVENTION," insert the following:

Legends:

Figure 1:
Alignment of the sequences of the predicted protein precursor of the HHV-8 IL-6 gene with human and mouse IL-6. Amino acieds identical in all three proteins are indicated by an asterisk, cysteine residues involved in disulfide bridging are marked with an arrowhead. Upper case letters symbolizse amino acids conserved according to Dayhoff criteria.

Figure 2:
Nucleic acid sequence encoding v-IL-6 and corresponding amino acid sequence.

Page 3, line 18 (under part d) delete "b1" and replace therefor with "b." under

Page 4, line 11 (under part i) replace "conditions and encoding functionally active v-IL-6 shall be comprised." With "conditions and may comprise sequence that encodes functionally active v-IL-6."

Page 5, line 20 (under part r) replace "auxilliary" with "auxiliary."

Page 6, line 13 (under part v) replace "hemopoetic" with "hemopoietic."

Page 6, line 18, replace "california" with "California."

Page 7, line 5, add "7: 4068-4077 (1990)." to the end of this line, so the entire line reads "Miles, S.A. et al.: Proc.Natl. Acad. Sci. U.S.A. 87: 4068-4072 (1990)."

Page 7, line 6, add "0: 969-975 (1994)." to the end of this line, so the entire line reads "Masood, R. et al.: AIDS Res. Hum. Retroviruses 10: 969-975 (1994)."

Page 7, line 10, add "(1994) to the end of this line.

**Marked up Claims to Show Amendm nts**

1. Viral interleukin-6 (v-IL-6), [which can be] obtained by recombinant expression of the DNA of [HHV-8] human herpes virus type 8 ("HHV-8") in an isolated cell.

2. [A] An isolated polypeptide[, which can be] obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence SEQ ID NO:2[displayed in fig. 2] in an isolated cell.

3. A polypeptide having the amino acid sequence SEQ ID NO: 2[displayed in FIG. 2].

4. A fragment of v-IL-6[, having the capability of binding to] that binds an interleukin-6 [1L-6] receptor and comprises the amino acid sequence (residues 87-105 of SEQ ID NO: 2) GFNE[ts]TSCLkKL[ad]ADGFFEFE.

5. A fragment as claimed in claim 4, consists [which] essentially of [comprises] the amino acid sequence (residues 87-105 of SEQ ID NO:2) GFNE[ts]TSCLkKL[ad]ADGFFEFE.

6. A fragment as claimed in claim 4 [or 5], which binds to a human IL-6 receptor.

8. A fragment obtained from [Fragments of] the v-IL-6 [as claimed in] of claim 1, [or the polypeptide as claimed in claim 2 or 3, characterized in that it is they able to] that can competitively inhibit the biological activity of IL-6 in a suitable assay system wherein the fragment binds to the receptor.

9. An isolated nucleic acid molecule and coding [essentially] of the sequence of SEQ ID NO:1 and coding for v-IL-6 [as claimed in claim 1] , which is obtainable by

recombinant expression of the DNA of human herpes virus type-8 (HHV-8) in an isolated cell.

10. An isolated nucleic acid molecule consisting of the sequence of SEQ ID NO:2 and coding for [the] a polypeptide [as claimed in claim 2] , which is obtainable by recombinant expression of the DNA of HHV-8 and which comprises the amino acid sequence SEQ ID NO:2.

11. An isolated nucleic acid having the nucleotide sequence of SEQ ID NO:1 of [displayed in fig. 2].

12. An isolated nucleic acid molecule, hybridizing under stringent conditions to the nucleic acid as claimed in [one or more of the claims 9 to] claim 11, encoding functional v-IL-6, wherein the nucleic acid encodes functional v-IL-6.

16. A [T]testkit for the detection of v-IL-6 DNA or RNA, comprising a nucleic acid [acic] molecule consisting of the sequence of SEQ ID NO:1 as claimed in claim 11 [one or more of the claims 9 to 12].

18. A [pharmaceutical] composition [medicament] comprising as an active ingredient [v-IL-6 as claimed in claim 1 and/or] the polypeptide as claimed in claim 2 [or 3, and/or mutants and variants of v-IL-6 as claimed in claim 7, and/or fragments of v-IL-6 as claimed in claim 4-6 or 8] and a pharmaceutically acceptable carrier.

19. A [pharmaceutical ] composition [medicament] comprising as an active ingredient the nucleic acid as claimed in [one or more of claims 9 to 12] claim 11 and a pharmaceutically acceptable carrier.

20. A cell culture growth medium, comprising [as an additional active ingredient] v-IL-6 as claimed in claim 1, [or the polypeptide as claimed in claim 2 or 3, or mutants

and variants as claimed in claim 7, or fragments as claimed in claim 8, or mixtures of these substances].

28. A fragment of a polypeptide that is obtainable by recombinant expression of the DNA of HHV-8 in an isolated cell and which comprises the amino acid sequence of SEQ ID NO:2 [of Fig. 2].

34. A method of culturing cells[, comprising adding to a cell culture comprising said cells a cell growth-stimulating amount of] using v-IL-6.

35. The method of claim 34, wherein said cells are selected from the group consisting of ß-lymphocytes, hybridomas, hemopoietic and endothelial cells.

TITLE OF THE INVENTION

# VIRAL INTERLEUKIN-6

## **CROSS-REFERENCE** TO RELATED APPLICATIONS

This is a national stage filing from Priority Application PCT/EP96/ 03199, filed July 19, 1996.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

## BACKGROUND AND SUMMARY OF THE INVENTION

### 1. Field of the Invention

The invention relates to diagnosis and treatment of diseases such as kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma and relates more particularly to viral interleukin 6 for the diagnosis and treatment of human disease.

Kaposi's sarcoma (KS), a multifocal proliferative lesion of uncertain pathogenesis, is highly prevalent among homosexual AIDS patients. Studies with biopsy materials and cultured cells have indicated an important role of growth factors and cellular cytokines, such as basic fibroblast growth factor, interieukin-1ß, platelet derived growth factor, interleukin-6 (IL-6), and oncostatin M for the proliferation of spindle cells in KS[1,2]. Several groups found indication for the expression of interleukin-6 (IL-6) receptors in AIDS-KS cells[3] and derived spindle cell lines[4]. As epidemiological evidence had suggested that an infectious agent other than HIV may also be involved in KS pathogenesis, it stirred considerable interest when Chang and colleagues[5] found DNA sequences of a novel herpesvirus in AIDS-KS tissues. Meanwhile, DNA of this virus was consistently found in all epidemiological forms of KS. The new virus, termed human herpesvirus 8 (HHV-8), shows marked sequence homology to herpesvirus (h.) *saimiri*, the prototype of $\gamma_2$-herpesviruses; thus HHV-8 appears to be the first human

member of $\gamma_2$-herpesviruses (genus rhadinovirus). Cloning HHV-8 DNA from KS tissues and sequencing indicates a genome organization that is generally collinear to *h. saimiri*[6].

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figur 1:**

Alignment of the sequences of the predicted protein precursor (SEQ ID NO: 2) of the HHV-8 IL-6 gene with human (SEQ ID NO: 3) and mouse IL-6 (SEQ ID NO: 4). Amino acids identical in all three proteins are indicated by an asterisk, cysteine residues involved in disulfide bridging are marked with an arrowhead. Upper case letters symbolize amino acids conserved according to Dayhoff criteria.

Figure 2: Nucleic acid sequence encoding v-IL-6 (SEQ ID NO: 1) and corresponding amino acid sequence (SEQ ID NO: 2).

## DETAILED DESCRIPTION OF THE INVENTION

In the course of these studies we surprisingly found, adjacent to a dihydrofolate reductase gene, an open reading frame (ORF) with the coding capacity for a 204 amino acid polypeptide with marked homology to mammalian IL-6 (P-value for homology searches with NCB/-BLAST: $P \leq 10\text{-}18$; percent identity/similarity to human IL-6: 24.74%/ 46.91%; to murine: 24.23%/ 47.94%; to porcine: 25.97%/ 52.91%; to bovine: 24.60%/ 49.73%; all alignments were calculated with the GCG software "GAP").

The viral gene product (v-IL-6) has conserved all 4 cysteine residues that are known to be involved in IL-6 disulfide bridging, and it shows a characteristic signal peptide of 19 to 22 amino acids (fig. 1). The area involved in binding of human IL-6 to its receptor has been mapped to the middle of the protein by two groups [7,8,9]. Ehlers et al. showed that amino acids 105 to 123 of the human IL-6, as shown in fig. 1 (GFNEEtCLVKlitGLLEFE)(residues 105-123 of SEQ ID NO:3), are involved in receptor binding. Most remarkably, this region is highly conserved in v-IL-6 (GFNEtsCLkKLadGFFEFE)(residues 87-105 of SEQ ID NO: 2). Identity and similarity of v-IL-6 to the receptor binding region of human IL-6 are 58% and 74%, respectively (fig. 1). This is almost identical with the degree of conservation that can be observed in this receptor binding area of human IL-6 to murine IL-6. As both human IL-6 and murine IL-6 are able to bind to the receptor of the other species (murine IL-6 and human IL-6, respectively), it is likely that v-IL-6 is also able to bind to the human and the murine IL-6 receptor.

Rhadinoviruses frequently acquire genes from their host cell[10]. This HIV-8 ORF however, is the first known example of a viral IL-6 structural homologue. Up to now all cell-homologous genes of rhadinoviruses that have been tested were functional; non-functional genes would most likely have been lost in viral evolution. Thus, the conservation of essential IL-6 features makes it highly suggestive that v-IL-6 is functional in normal HHV-8 replication or persistence. Since models of paracrine growth stimulation of spindle cells by cytokines, including IL-6 and the related oncostatin M, have been proposed for KS pathogenesis, the finding of the v-IL-6 gene in HHV-8 lends support to the hypothesis that HHV-8 is causally related to this multifocal proliferation.

The present invention therefore relates to:

a) Viral interleukin-6 (v-IL-6), which can be obtained by recombinant expression of the DNA of HHV8.

b) A polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.

c) A fragment of v-IL-6, having the capability of binding to an IL-6 receptor and comprising the amino acid sequence GFNEtsCLkKLadGFFEFE (RESIDUES 87-105 of SEQ ID NO: 2).

d) A fragment as defined in b, which essentially comprises the amino acid sequence GFN EtsCLkKLadGFFEFE (residues of SEQ ID NO: 2).

e) A fragment as defined in c or d, which binds to a human IL-6 receptor.

f) A polypeptide having the amino acid sequence displayed in fig. 2.

g) Mutants and variants of v-IL-6 or of the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, which mutants and variants are obtained by conventional amino acid substitutions or deletions, with the proviso that these mutants and variants are functionally equivalent to v-IL-6.

h) Fragments of v-IL-6, or of the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, characterized in that they are able to competively inhibit the biological activity of IL-6 in a suitable assay system.

i) An isolated nucleic acid coding for v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2. A preferred embodiment is the nucleic acid having the nucleotide sequence of fig. 2. Furthermore, an isolated nucleic acid, hybridizing to the above mentioned nucleic acids under stringent conditions and may comprise a sequence that encodes functionally active v-IL-6.

k) Monoclonal or polyclonal antibodies directed against v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.

l) Testkit for the detection of v-IL-6 in a sample, comprising one or more of the above monoclonal or polyclonal antibodies.

m) Testkit for the detection of antibodies against v-IL-6 comprising v-IL-6 and/or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, and/or mutants and variants of v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2 and/or fragments of v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.

n) Testkit for the detection of v-IL-6 DNA or RNA, comprising a nucleic acid which codes for v-IL-6, or which hybridizes to the aforementioned nucleic acid and encodes functionally active v-IL-6.

o) A medicament comprising as an active ingredient a monoclonal antibody or polyclonal antibodies directed against v-IL-6, or a polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants, variants or fragments of v-IL-6 or the aforementioned polypeptide. In another embodiment, the medicament may comprise as an active ingredient a nucleic acid encoding v-IL-6.

p) A cell culture growth medium, comprising as an active ingredient v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2 , or mutants, variants or fragments of v-IL-6 or the aforementioned polypeptide.

q) A process of manufacturing v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants and variants, or fragments of v-IL-6 or the aforementioned polypeptide.

r) A process of manufacturing a medicament, wherein the active ingredient is combined with suitable excipients and/or other auxiliary compounds according to common knowledge of those skilled in the art.

s) A process of manufacturing a medicament comprising as an active ingredient monoclonal or polyclonai antibodies directed against v-IL-6, or a polypeptide comprising v-1L-6, or mutants, variants or fragments of v-IL-6, or a nucleic acid encoding v-IL-6 for the treatment of kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma.

t) A process of diagnosing an HHV-8 infection comprising the in vitro detection of v-IL-6 antigen, v-IL-6 DNA, v-IL-6 RNA or antibodies against v-IL-6.

u) A process of diagnosing the HHV-8 associated disorders kaposi sarcoma, Castleman's disease or body cavity based lymphomas (BCBL) through the diagnosis of an HHV-8 infection as described above.

v) A process of growing cells in culture, characterized in that v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants and variants, or fragments of v-IL-6 or the aforementioned polypeptide, or mixtures of these compounds are contained in the growth medium. In a preferred process these cells are B-lymphocytes, hybridomas, hemopoietic cells or endothelial cells.

The sequence shown in fig.2 was generated by first subcloning shotgun fragments of lambda clone G16 into commercially available plasmid pBS KS- (Stratagene, San Diego, California). Resulting plasmids were purifed using a commercially available kit (Qiagen, Hilden, Germany) and sequenced on an automated sequencing system (A377, Applied Biosystems GmbH, Weiterstadt, Germany) using the recommendations of the manufacturer. The sequence was determined on both strands, using standard primers for shotgun clones, and gene specific primers for further analysis. In addition to showing the coding sequence of the interleukin-6 homologue of human herpesvirus 8, the deduced amino acid sequence, in one and three letter code, is shown in the sequence listing below.

The present invention is further described in the claims.

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